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Pro-Active Plants

Exploiting photosystem I as a light-driven reductase

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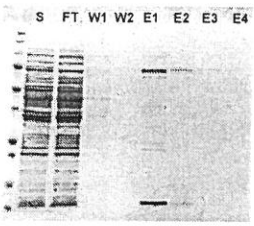
Introduction

Photosystem I carries out light-mediated electron transfer to NADP+ using the soluble electron carriers ferredoxin (Fd) and ferredoxin NADP+ oxidoreductase (FNR) resulting in the formation of reducing power in the form of NADPH. FNR interacts with Fd through a FAD cofactor binding domain. A structurally similar FAD domain is found in the membrane bound cytochrome P450 oxidoreductase (CPR). CPR mediates electron transport from NADPH via the one electron carriers FAD and FMN to the heme of membrane bound cytochrome P450. By taking advantage of the similar FAD domains, it should be possible to engineer electron transfer from PSI to P450 and to combine the catalytic properties of the two membrane complexes which in nature are localized in the chloroplast and endoplasmatic reticulum, respectively.

Expression and purification of ΔN-CPR from *Sorghum bicolor*



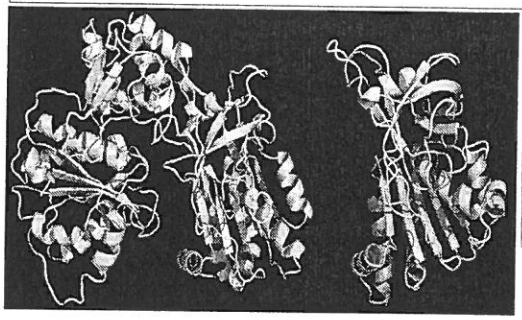
The *Sorghum bicolor* CPR gene was cloned without the membrane anchor into an E. coli expression vector (pET-15b) resulting in a fusion protein with a C-terminal His-tag.

The his-tagged protein was expressed in *E. coli* as a soluble protein. It was purified using Ni-NTA agarose beads and was found to be in an active conformation based on its ability to reduce cytochrome *c*.

S: Start material
FT: Flow-through
W: Wash 1 and 2
E: Elution 1 to 4 in 250mM imidazole

Model structure of *Sorghum bicolor* CPR

Comparison of the protein structure of *Sorghum bicolor* CPR with structure of FNR from the cyanobacterium Anabaena



Pale Green residues: FMN binding domain

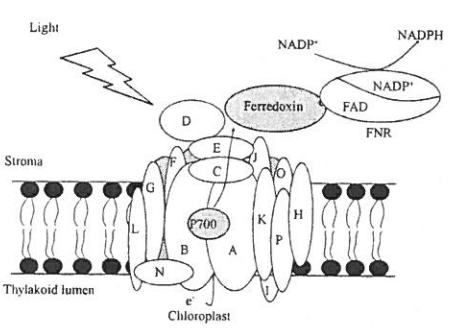
Light Blue residues: NADPH binding domain

Yellow residues: FAD binding domain

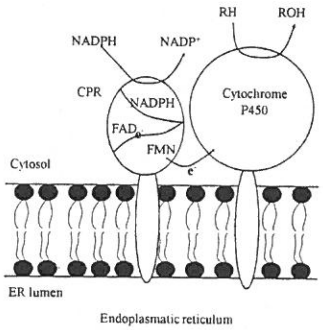
White residues: Linker domain

Based on the structurally similar FAD/NADPH-binding domains of CPR and FNR, it is seems plausible that CPR can bind to Fd and thereby generate a novel electron transfer between two otherwise non-connected systems. The FAD and NADPH domains of CPR forms along with the linking domain (white) a crescent shaped protein in the center of which the Fd protein is expected to bind and deliver its electrons. The FMN binding domain seems to obstruct docking of Fd but it has been postulated that the FMN domain is mobile and that movement of this domain would coincide with FMN release/binding [1].

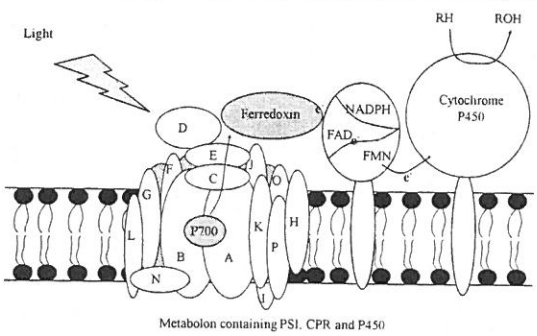
Combining photosystem I and cytochrome P450



Photosystem I is a multisubunit protein complex located in the thylakoid membranes of green plants. It is extremely efficient in its utilization of light for electron transport from plastocyanin on the luminal side to ferredoxin on the stromal side of the thylakoid membranes. Following detergent solubilization, PSI can be isolated from thylakoid preparations by ultracentrifugation in a sucrose gradient.



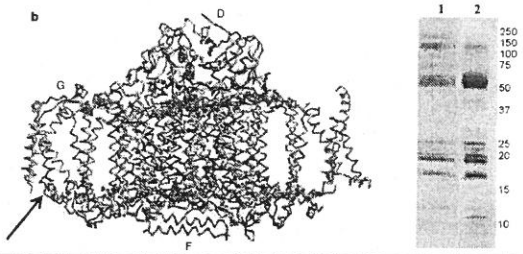
Cytochrome P450s are membrane bound proteins processing an unequalled catalytic versatility, although the most common reactions catalyzed can be classified as monooxygenations. CPR donates electrons to P450 through the FMN domain,



Metabolon containing PSI, CPR and P450

The formation of a metabolon containing PSI and P450 will generate a nanoscale fuel cell providing reducing power for the P450s catalytic activity. The protein complexes will be immobilized on nanowires. This will ensure an efficient electron transfer from the nanowires to the PSI reaction center. The complex formed will be able to utilize light energy for the synthesis of complex organic molecules .

Purificartion of His-tagged photosystem I



A quick method for purification of PSI has been developed. A His-tag was introduced in the gene encoding the PsaG subunit in Arabidopsis thaliana [2]. See approximate location of the His-tag in the above structure of the PSI complex (left). A 1-hour procedure involving solubilization of the thylakoid membranes with beta-dodecylmaltoside and purification using Ni-NTA agarose yielded fairly pure PSI: lane 1 reveals the protein composition of the His-tag purified PSI and lane 2 reveals the PSI complex composition using a 20-hour procedure using sucrose gradients. Apparently, the only major difference is the loss of PsaN with the His-tag procedure (verified by immunoblotting, data not shown).

Discussion

The objective of this project is the formation of a metabolon encompassing both PSI and cytochrome P450 as a functional unit that can be used to hydroxylate desired substrates based on a light driven electron transfer from PSI.

The electron transfer between PSI and cytochrome P450 depends on the ability of CPR to interact with Fd. FNR-Fd interactions is mediated by the FAD binding domain of FNR. Based on the structural similarity of the FAD binding domain in FNR and CPR, it seems likely that CPR will be able to accept electrons from Fd. One complication could be the positioning of the FMN binding domain in CPR that blocks access to the FAD binding domain for Fd, but the FMN domain appears to be mobile.

If the interaction is very weak between Fd and CPR, a possible way to enhance the electron transfer would be to generate fusion proteins of Fd and CPR to increase the frequency of constructive protein-protein interactions.

The his-tagged PSI will enable both quick purification and also a way to immobolize the PSI complex on surfaces.

References

[1] Wang et al. (1997). Three-dimensional structure of NADPH-cytochrome P450 reductase: prototype for FMN- and FAD-containing enzymes. Proc Natl Acad Sci U S A 94(16): 8411-6
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